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The effect of *Centella asiatica* L. Urban. and *Curcuma longa* L. extracts combination in improving memory performance in stroke model rats and its acute toxicity

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ABSTRACT

Post-stroke cognitive impairment involves memory, visuoconstructional, and spatial dysfunctions. *Centella asiatica* L. Urban. and *Curcuma longa* L. are both well-known herbs in South and South-East Asia countries that are believed to possess neuroprotective properties as memory enhancers. This study aimed to investigate the effects of *C. asiatica* L. Urban. and *C. longa* L. extracts combination in improving memory performance in stroke model rats and its acute toxicity. Twenty-five Wistar rats underwent transient bilateral common carotid artery occlusion. Y-maze pre-test was performed 24 h after the occlusion. The rats were then divided into five groups i.e. Group 1 received NaCMC dilution, Group 2 received donepezil 0. 7 mg/kg BW/day, and Groups 3, 4, and 5 received the extracts combination with dose of 59; 118; and 236 mg/kg BW/day, respectively. Y-maze post-test was performed 24 h after the last dose had been given. Spontaneous alternation behavior was used as the indicator of working memory measurement. The fixed-dose method according to OECD Guideline was used to estimate the LD_{50} in acute toxicity testing on Wistar rats. There was a significant difference in the delta spontaneous alternation percentage among groups tested ($p<0.05$). Group 1 had significant difference with any other group (compared to Groups 2 to 5; $p<0.05$). No significant difference could be found between groups of the extracts combination and donepezil group suggesting that the effect is not more inferior nor dose-dependent in improving memory performance. The extracts combination at a single dose of 2000 mg/kg BW did not show related signs of toxicity or mortality in any of the rats during the 14-day observation period. In conclusion, the *C. asiatica* L. Urban. and *C. longa* L. extracts combination can improve performance of memory on stroke model animal. According to Globally Harmonized Classification System, the extracts combination can be classified as Category 5/unclassified.

ABSTRAK

Gangguan kognitif pasca stroke melibatkan disfungsi memori, visuokonstruksi, dan spasial. *Centella asiatica* L. Urban. dan *Curcuma longa* L. keduanya dikenal luas di negara-negara Asia Selatan dan Tenggara sebagai tumbuhan yang diyakini memiliki sifat neuroprotektan untuk meningkatkan daya ingat. Penelitian ini bertujuan untuk mengkaji efek kombinasi ekstrak *C. asiatica* L. Urban. dan *C. longa* L. dalam meningkatkan kinerja memori pada tikus model stroke dan toksisitas akutnya. Dua puluh lima tikus Wistar menjalani oklusi arteri karotis komunis bilateral sementara. Pre-tes Y-maze dilakukan 24 jam setelah oklusi. Tikus dibagi menjadi lima kelompok yaitu Kelompok 1 mendapat larutan NaCMC, Kelompok 2 mendapat donepezil 0,7 mg/kg BB/hari, dan Kelompok 3,

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4, dan 5 mendapat kombinasi ekstrak dengan dosis berturut-turut 59; 118; dan 236 mg/kg BW/hari. Pos-tes Y-maze dilakukan 24 jam setelah dosis terakhir diberikan. Perilaku alternasi spontan digunakan sebagai pengukuran fungsi kognitif. Metode dosis tetap menurut petunjuk OECD digunakan untuk memperkirakan LD50 dalam uji toksisitas akut oral pada tikus Wistar. Terdapat perbedaan nyata antara kelompok dalam persentase alternasi spontan delta ($p < 0,05$). Kelompok 1 memiliki perbedaan yang signifikan dengan kelompok lain (dibandingkan Kelompok 2-5, $p<0,05$). Tidak ada perbedaan yang signifikan yang dapat ditemukan di antara kelompok kombinasi ekstrak dan dengan kelompok donepezil, hal ini menunjukkan bahwa efeknya lebih rendah dan kemungkinan tidak tergantung pada dosis. Kombinasi ekstrak dosis tunggal 2000 mg/kg BB tidak menunjukkan gejala toksisitas dan kematian pada tikus selama 14 hari pengamatan. Dapat disimpulkan, kombinasi ekstrak *C. asiatica* L. Urban. dan *C. longa* L. dapat memperbaiki perbaikan memori pada tikus model stroke. Menurut *Globally Harmonized Classification System*, kombinasi ekstrak dapat digolongkan sebagai Kategori 5/unclassified.

INTRODUCTION

Cognitive decline, associated stroke, stress, and other neurodegenerative diseases is a major health issue. Cognitive slowing is a common finding in post-stroke patients, particularly a distinctive slowness in information processing.[1,2] Post-stroke cognitive impairment is defined as deficit in cognitive function that involves at least one cognitive domain, either attention, memory, executive function, perception, or language. This impairment is not typically domain-specific and not consistently influencing certain cognitive domains.[3-7] A study reported that impairments in processing speed, attention, and executive function were frequently found in elderly stroke patients without dementia.[3] However, impairments in long-term (episodic) memory[4,5] as well as visuoconstructional and spatial functions[6,7] were also reported as the most common findings in post-stroke cognitive decline.

Centella asiatica L. Urban., also known as pegagan or gotu kola, is widely known and found in South and South East Asia. Its use as memory enhancer is increasingly common in traditional herbal medicine in India, China, Africa, and South East Asia.[8] Its primary main chemical constituents that are widely studied are asiatocosides, asiatic acid, madecoside, and madasiatic acid.[9] Not only widely studied as memory enhancer, *C. asiatica* L. Urban. is also recognized with its effects as anti-inflammation, anti-oxidants, antiapoptotic, neuroprotective, hepatoprotective, antidepressant, anticonvulsant, immunoprotection, antitumor, antiviral, antibacterial, insecticidal, an antifungal.[9] As neuroprotective agent and memory enhancer, *C. asiatica* L. Urban. leaf extracts have been reported in animal and human studies in our country with promising.[10,11] *Centella asiatica* L. Urban. and asiaticocide were reported to be well tolerated, with up to 1 g/kg BW of asiaticoide did not induce any toxic effects.[12] In Wistar rats, the *C. asiatica* L. Urban.dose up to 10,000 mg/kgBW once and 1,000 mg/kgBW for 90 days caused no toxic effects in acute and sub-chronic use, respectively.[13]

Curcuma longa L., or also known as curcumin or turmeric, is grown for food's spices and coloring agent in South and South East Asia's cuisine. Its main chemical constituents, called curcuminoids that consisted of curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin.[14] Curcuminoids were previously reported to possess the properties of as protectants for brain, liver, inflammation, cancer growth, as well as analgesic, antiproliferative, antidiabetic, antithrombotic, antidiarrheal effects.[15] Studies have shown that curcumin showed its neuroprotective effects in cognitive impairment caused by chronic stress or Alzheimer's Disease by normalizing corticosterone response and reducing amyloid, respectively.[16-20] In BALB/c mice, single oral administration of ethanolic extract of *C. longa* L. at the dose of 2,000 mg/kg BW did not exert any toxic

effects.[21] To our knowledge, there were no previous reports regarding the acute toxicity and neuroprotective effects of oral *C. asiatica* L. Urban. and *C. longa* L. extracts combination in improving memory performance in stroke models rats.

MATERIALS AND METHODS

Preparations of *C. asiatica* L. Urban. and *C. longa* L. extracts combination

Standardized *C. asiatica* L. Urban. leaves' extract was obtained from local Herbal Medicine Industry, PT Borobudur, Semarang, whereas standardized *C. longa* L. rhizome extract was obtained local Herbal Medicine Industry, PT Phytochemindo, Bogor. Capsule preparation containing the both was produced by local Herbal Industry, PT Swayasa Prakarsa, Yogyakarta according to the Good Manufacturing Practice Guideline for Herbal Medicine (GMPGHM). Each capsule consisted of 187. 5 mg *C. asiatica* L. Urban. extract and 100 mg *C. longa* L. extracts.

Stroke model rats study of *C. asiatica* L. Urban. and *C. longa* L. extracts combination

Animal and experimental design.

This was a quasi experimental that use pre- and post-controlled group design on stroke model rats after oral administration of *C. asiatica* L. Urban. and *C. longa* L. extracts combination. Twenty-five healthy male *Rattus norvegicus* of the Wistar strain, 150 – 200 g in weight, 8 – 12 weeks-old, were obtained from Experimental Animal Care Unit (UPHP) of Universitas Gadjah Mada. Each rat was determined as healthy according to its physical state, i.e. clean, without sticky or wet bristles, mobility active, and appropriate cycle of drinking, eating, and sleeping. To ensure the rats' circadian rhythms, a group consists of two rats were housed inside a glass cage, under the following conditions: room temperature 25-30˚C, 50%-60% humidity, a dark-light cycle of 12:12 h, and fed with a standard pellet diet and water ad libitum.

All rats underwent transient bilateral common carotid artery occlusion (tBCCAO) to create stroke model rats. The surgical procedure was performed as previously reported Handayani *et al*.[22] with 10 min occlusion. Briefly, 100 mg/kgBW ketamine was injected intraperitoneally for anesthesia. After aseptic procedure, midline vertical incision was made in anterior neck, then submandibular gland and sternocleidomastoid muscles were dissected to expose bilateral CCA (common carotid artery), and both CCAs were clamped using microvascular clamps for 10 min.

The rats were then randomized into five groups equally: (1) Group 1 is stroke model rats with only oral administration of sodium NaCMC 0. 5%; (2) Group 2 is stroke model rats with oral administration of donepezil 0. 7 mg/kg BW; (3) Group 3-5 are stroke model rats with oral administration of the extracts combination at dose of 59; 118 and 236 mg/kgBW, respectively. The oral administrations of the drugs and or diluent preparations were given daily for fourteen consecutive days using gastric tube.

Prior to tBCCAO procedure, all rats were acclimatized to the environment and familiarized with the Y-maze for five min for seven consecutive days. Twenty-four hours after the tBCCAO procedure, each rat underwent spatial memory and behavioral test in Y-maze as the pre-test. On the following day, each rat started receiving oral drug preparations according to the randomized group for fourteen consecutive days. One day after the last preparation's administration was given, each rat underwent the second test using Y-maze to evaluate its working memory performance (post-test). The detailed experimental designs were described in FIGURE 1. The study was approved by the Medical and Health Research Ethic Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta (No. KE/FK/0600/EC/2017).

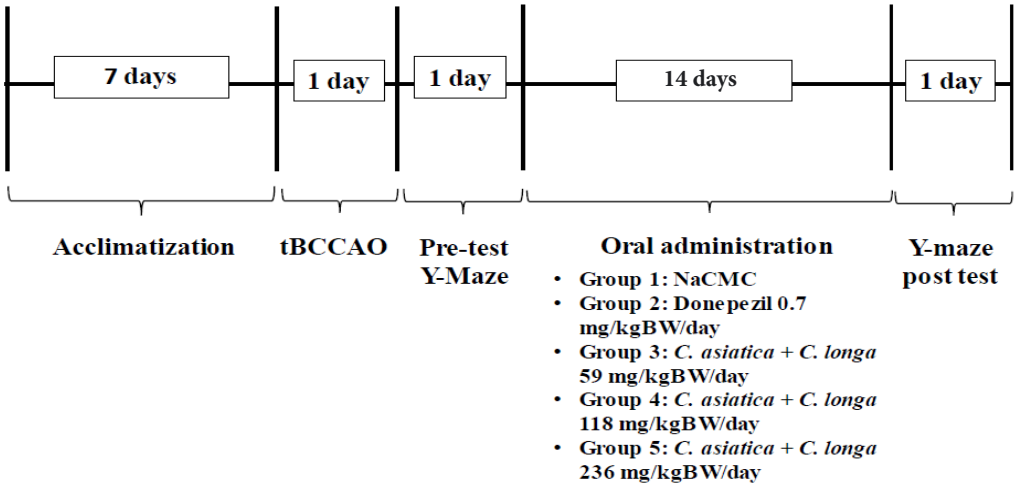

FIGURE 1. Experimental design. All rats were acclimatized in Y-maze for seven consecutive days before the procedure of underwent tBCCAO. Y-maze task for pre-test was performed twenty-four hours after tBCCAO. The oral treatments for five different groups were given for fourteen consecutive days. Twenty-four hours after the last given dose, Y-maze task for post-test was performed. tBCCAO: transient bilateral common carotid artery occlusion, NaCMC: sodium carboxymethylcellulose.

Y-Maze test.

The Y-maze apparatus is a three-arms maze made of sturdy plastic painted in white. Each arm has 50 cm long, 10 cm wide, and 20 cm height, intersected at 120°. Each rat was firstly located at the end of an arm and allowed to explore the maze freely for five min with pellets placed at the end of every arm for the rewards. A video camera was placed above the Y-maze apparatus to record the behavioral test. Using the recorded video, working memory was observed by a blinded researcher to determine the exploration of each rat into the three arms of Y-maze. An entry into one arm was noted if all four paws of the rat were inside the arm. The Y-maze task was initiated and tested in the same period of time during acclimatization as well as pre- and post-test, in the morning started at 8 a.m.

Spontaneous alternation behavior was used as the working memory performance measurement in this study representing the retention of the rat's spatial memory and its nature to explore new place. Spontaneous alternation was defined as entries to three different arms consecutively. The spontaneous alternation percentage was calculated with the following formula: [number of spontaneous alternations/number of arm visits in total] – 2. A low percentage of spontaneous alternations represented poor working or spatial memory.[23]

Acute oral toxicity study of *C. asiatica* L. Urban. and *C. longa* L. extracts combination

The oral toxicity study of the *C. asiatica* L. Urban. and *C. longa* L. extracts combination was performed using fixed-dose method according to Organization for Economic Co-operation and Development (OECD) Guideline Testing of Chemicals.[21] Eleven female rats, *Rattus norvegicus* of Wistar strain 12 weeks old weighing 120-200 grams were used for the test. The dose of 300 mg/kgBW was selected for the initial dose. In the primary study, one rat that received the initial dose did not show any symptoms of toxicity. Furthermore, the dose of 2000 mg/kgBW also did not show any toxicity symptoms either. Therefore, the toxicity study was continued using another

four different rats with the extracts combination at dose of 2000 mg/kg BW. As control, another five rats that were given NaCMC solution. After the dose had been given, each rat was observed for physical sign and any possibility of toxicity in 24-h and followed up to 14 days. Body weight were measured on day-1, 7, and 14. At the end of the observation, the rats were terminated. The brain, heart, liver, lungs, stomach, intestines, kidneys, and ovaries were dissected out and weighed for macroscopic examination. Histopathological examinations of these organs were performed using hematoxylin-eosin staining and compared between groups. The polyherbal formula was then ranked and categorized according to the Globally Harmonized System (GHS) for the classification of chemicals that may induce acute toxicity effect.[21]

Statistical analysis

For the acute toxicity study, the rats' body weights and main organs' weights were presented as mean ± SD. The difference between groups was analyzed using t-test following the normal data distribution testes using Saphiro Wilk. For the Y-maze behavioral test, the delta spontaneous alternation percentage between the post-test and pre-test in Y-Maze task before and after intervention was used to see the increase in spatial working memory of the rats. The normality of the data distribution was tested using Saphiro Wilk. Following the normal data distribution, ANOVA test was assigned to analyze the difference in the mean of delta spontaneous alternation percentage between groups. Furthermore, post-hoc analysis was performed to distinguish which groups showed the significant differences. All statistical analyses were conducted using SPSS version 23.

RESULTS

Stroke model rats study

All twenty-five animals survived from the beginning to the end of the study and completed the pre- and post-test in the Y-maze task. The mean delta spontaneous alternation percentage between post- and pre-test is presented in TABLE 1.

TABLE 1. The percentage (mean ± SEM) of delta spontaneous alternation between post- and pre-test in five groups

Group	n	Delta spontaneous alteration percentage	p
NaCMC	5	-51.83 ± 5.15	
Donepezil 0.7 mg/kgBW/day	5	5.33 ± 10.38*	
C. asiatica + *C. longa* 59 mg/kgBW/day	5	7.75 ± 12.21*	0.037
C. asiatica + *C. longa* 118 mg/kgBW/day	5	5.92 ± 7.88*	
C. asiatica + *C. longa* 236 mg/kgBW/day	5	27.89 ± 3.08*	

*) p value <0. 05 compared to Group 1 using Post Hoc LSD

The highest improvement in the spontaneous alternation percentage was seen in Group 5 while the lowest improvement was seen in Group 1. ANOVA test showed significant difference between groups in the delta spontaneous alternation percentage after and before the drugs preparation's administration for two weeks ($p<0.05$). Furthermore, post-hoc analysis revealed that Group 1 had significant difference with any other group. This might suggest that the drugs preparation, namely donepezil as well as *C. asiatica* L. Urban. and *C. longa* L. extracts, might have benefit in improving the spatial working memory in rats, as compared to placebo. No significant difference could

be found between groups of *C. asiatica* L. Urban. and *C. longa* L. extracts with donepezil group, suggesting the effect of *C. asiatica* L. and *C. longa* L. extracts is not more inferior compared to donepezil in improving spatial memory in rats. No significant difference could be found between groups of *C. asiatica* L. Urban. and *C. longa* L. extracts with three different doses, suggesting that the effect might not be dose-dependent.

Acute oral toxicity study

We firstly observed one rat that was given an initial dose 300 mg/kg BW of *C. asiatica* L. Urban. and *C. longa* L extracts combination. The observations did not show any physical signs and symptoms as well as any possibilities of toxicity on the first 30 min, 1 h, 24 h, until 14 days. Interestingly, observation to a higher dose, 2,000 mg/kgBW *C. asiatica* L. Urban. and *C. longa* L. extracts combination did not show any physical signs nor symptoms as well as any possibilities of toxicity with the same length observation period. Body weight was measured and compared between rats' groups. The mean body weight of these rats did not show any significant difference at day-0,7, and 14 suggesting that the weight gain is normal up to 14 days observation (TABLE 2).

TABLE 2. Body weight of female rats (mean ± SD) receiving single dose of 2000 mg/kg BW of *C. asiatica* L. Urban. and *C. longa* L. extracts combination

Treatment	Day-0	Day-7	Day-14	p_1
NaCMC	182±16. 97	205. 5±12. 02	211±11. 31	0. 094
C. asiatica and *C. longa* (2,000 mg/kg BW)	177. 80±18. 07	201. 20±14. 66	211. 50±14. 46	0. 063
p_2	0. 790	0. 732	0. 967	

p1: paired t test; p2 : independent t test; NaCMC: sodium carboxy methyl cellulose

Macroscopically, the examinations of the brain, heart, liver, lungs, stomach, intestines, kidneys, and ovaries did not show any abnormality between groups that received NaCMC and those received *C. asiatica* L. Urban. and *C. longa* L. extracts combination at dose of 300 and 2000 mg/kgBW (TABLE 3).

TABLE 3. Organ's weight of female rats (mean ± SD) receiving single dose of 2000 mg/kg BW of *C. asiatica* L. Urban. and *C. longa* L. extracts combination

Organ	NaCMC	*C. asiatica* and *C. longa* (2,000 mg/kg BW)	p
Heart	0. 003±0. 0002	0. 004±0. 000	>0. 05
Liver	0. 040±0. 0000	0. 038±0. 003	>0. 05
Lungs	0. 010±0. 0017	0. 009±0. 002	>0. 05
Pancreas	0. 005±0. 0008	0. 003±0. 000	>0. 05
Gastr	0. 012±0. 0013	0. 009±0. 000	>0. 05
Intestine	0. 089±0. 0081	0. 087±0. 001	>0. 05
Right kidney	0. 004±0. 0002	0. 004±0. 000	>0. 05
Left kidney	0. 004±0. 0000	0. 003±0. 000	>0. 05
Brain	0. 008±0. 0005	0. 007±0. 001	>0. 05
Ovaries	0. 005±0. 0013	0. 004±0. 001	>0. 05

Microscopically, no abnormality was found in the brain, heart, liver, lungs, stomach, intestines, kidneys, and ovaries between the groups that received NaCMC and *C. asiatica* L. Urban. and *C. longa* L. extracts combination at dose of 300 and 2000 mg/kgBW (FIGURE 2). According to these data, the lethal dose 50% (LD_{50}) of *C. asiatica* L. Urban. and *C. longa* L. extracts combination are > 2000 mg/kgBW. Global Harmonization System criteria for acute toxicity indicated that these extracts combination was categorized into category 5 or unclassified (2000 mg/kgBW < LD50 < 5000 mg/kgBW).

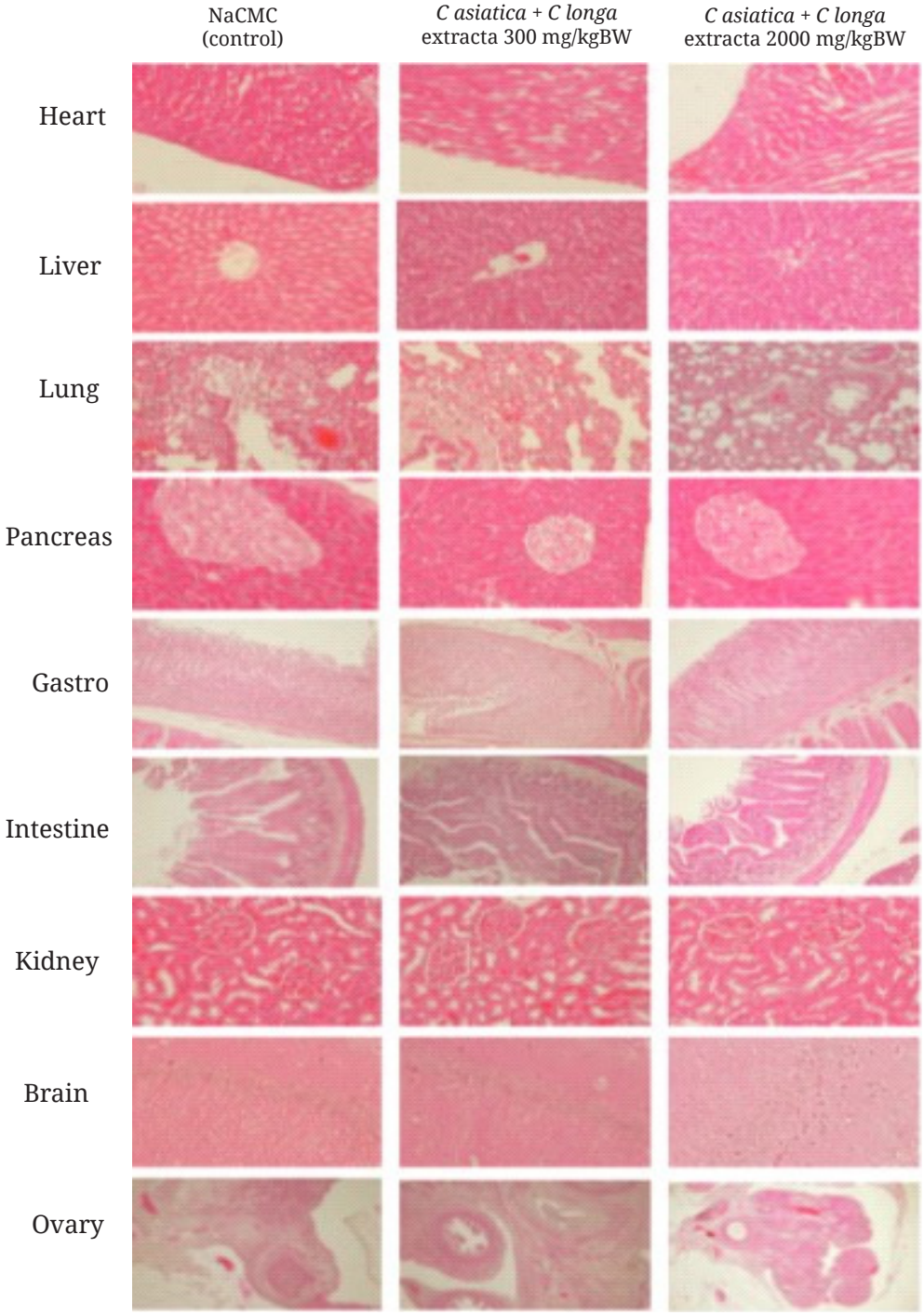

FIGURE 2. Histomorphology of hematoxylin-eosin stained samples of main organs showing no abnormalities between groups of NaCMC and C. asiatica L. Urban. + C. longa L. extracts combination at dose of 300 and 2000 mg/kgBW (400x magnification).

DISCUSSION

Cognitive impairment in post-stroke affects multiple cognitive domains. The domains of executive control and attention have primary role in performing complex cognitive function. These processes determine complex visuoconstructional and effective memoryfunction.Inhuman,impairments in long-term memory, executive functions, and visuoconstruction, or combinations of these three domains' deficit are more common compared to impairments in other subjects, e. g. abstract reasoning, language, reading, writing, and arithmetic abilities.[23]

Animal studies using rodents have abilities to show the memory performance in order to test their behavior by employing certain kind of maze. Y-maze is used to measure cognition ability, particularly memory function in rodents. Spontaneous alternation test is purposely used to measure the spatial working memory that can be assessed by allowing the mice or rats to move freely through all three arms of the Y-maze and is directed by the instinctive interest of rodents to search for the unvisited area.[24] A rat with intact working memory, suggesting a non-dysfunctional prefrontal cortex, will recognize the previously visited arms and display intention to enter another arm or less recently visited arm.

Our study showed that the combination of *C. asiatica* L. Urban. and *C. longa* L. extracts for fourteen days to improve the memory performance in stroke model rats. The mean delta spontaneous alternation percentage between post- and pre-test in Y-maze task of the treated groups (groups 2 – 5 vs group 1) showed significant difference. This phenomenon may suggest memory improvement after stroke might occur due to the given drugs. Furthermore, the doses of *C. asiatica* L. Urban. and *C. longa* L. extracts combination given in this current study could be considered safe since it did not exceed the acute nor the chronic toxicity level in rats.

Several studies showed *C. asiatica* L. Urban. and *C. longa* L. if given individually, were shown to increase memory performance as well as provide neuroprotection by up regulating BDNF expression.[25-27] Other study reported that *C. asiatica* L. Urban. may also improve memory function via reducing the expression of nitric oxide in rats.[26] However, studies using *C. asiatica* L. Urban. and *C. longa* L. extracts combination were not frequently found. A prior study using the combinations of several herbs *Tinospora cordifolia, C. asiatica* L. Urban., *Withhania somnifera, Mucuna pruriens*, and *C. longa* L. in composition ratio of 1:0. 5-1:1:1:2, respectively with the dose of 100 mg/kg BW/day chronically may be useful for the treatments of senile dementia by reducing the levels of oxidative stress.[28]

A recent study showed that a phytosomal formulation containing the combination of *C. asiatica* L. Urban. and *C. longa* L. may induce the increased expression of brain-derived neurotrophic factor (BDNF) as well as its downstream signaling pathway through interaction with TRKB receptor in prefrontal cortex of rats.[25] Furthermore, BDNF protein was upregulated in the neuronal dendrites as well as synapses in prefrontal cortex of rats.[29]

In human, several studies showed that BDNF level decreases in stroke patients[30] and stroke survivors with cognitive impairment.[31] In animals, BDNF expression in rats were found to be lower in hippocampal of post-stroke model[32] as well as post-stroke depression model.[33] Therefore, treatments targeting to increase BDNF expression is considered to ameliorate the neuronal injury and function after ischemic stroke.[32,34] Therefore, future study is necessary to elucidate whether BDNF is involved in the improvement of memory performance in post-stroke subjects after the administration of *C. asiatica* L. Urban. and *C. longa* L. extracts combination.

A toxicity study is mandatory in the use of novel drugs or herbs, either as monotherapy or polytherapy formula, for treating a disease. To our knowledge, this is the first study that reports the

acute toxicity of *C. asiatica* L. Urban. and *C. longa* L. extracts combination in rats. Our study revealed that the LD_{50} of *C. asiatica* L. Urban. and *C. longa* L. extracts combination was more than 2,000 mg/kg BW as proven physically, macroscopically, and microscopically. This suggested that the dose used in this study is safe.

CONCLUSION

In conclusion, oral administration of C. asiatica L. Urban. and C. longa L. extracts combination for 14 days can improve memory performance in stroke model rats demonstrated by the increase of the delta spontaneous alteration percentage after Y maze post test. Furthermore, the LD_{50} of the extracts combination is greater than 2,000 mg/kg BW that can be classified as Category 5/ unclassified.

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REFERENCES

1. Hostenbach J,Mulder T, van Limbeek J, Donders R, Schoonderwaldt H. Cognitive decline following stroke: a comprehensive study of cognitive decline following stroke. J Clin Exp Neurol 1998; 20: 503-17. https://doi. org/10. 1076/jcen. 20. 4. 503. 1471
2. Rasquin S, Lodder J, Verhey F. The association between psychiatric and cognitive symptoms after stroke: a prospective study. Cerebrovasc Dis 2005; 19(5): 309-16. https://doi. org/10. 1159/000084499
3. Ballard C, Stephens S, Kenny R, Kalaria R, Tovee M, O'Brien J. Profile of neuropsychological deficits in older stroke survivors without dementia. Dement Geriatr Cog Dis 2003; 16(1):52-6. https://doi. org/10. 1159/000069994
4. Tatemichi TK, Desmond DW, Stern Y, Paik M, Bagiella. Cognitive impairment after stroke: frequency, patterns, and relationship to functional abilities J Neurol Neurosurg Psychiatry 1994; 57:202-7. https://doi. org/10. 1136/jnnp. 57. 2. 202
5. Jaillard A, Naegele B, Trabucco-Miguel S, LeBas JF, Hommel M. Hidden dysfunctioning in sub acute stroke. Stroke 2009; 40: 2473-9. https://doi.org/10.1161/STROKEAHA.108.541144
6. Nys GMS, van Zandvoort MJE, de Kort PLM, Jansen BPW, Den Haal EHF, Kappelle LJ, *et al.* Cognitive disorders in acute stroke: prevalence and clinical determination. Cerebrovasc Di 2007; 23(5-6):408-16. https://doi.org/10.1159/000101464
7. Middleton LE, Lam B, Fahmi H, Black SE, Mcllroy WE, Stuss DT, *et al.* Frequency of domain-specific cognitive impairment in subacute and chronic stroke. Neuro Rehabilitation 2014; 34 (2): 305-12. https://doi.org/10.3233/NRE-131030
8. Mehla J, Gupta P, Pahuja M, Diwan D, Diksha D. Indian medicinal herbs and formulations for Alzheimer's Disease, from traditional knowledge to scientific assessment. Brain Sci 2020; 101(2): 964. https://doi.org/10. 3390brainsci10120964
9. Gohil KJ, Pattel JA, Gajjar AK. Pharmacological review on *Centella asiatica*: a potential herbal cure-all. Indian J Pharm Sci 2010: 72 (5):546-56. https://doi.org/10.4103/0250-474X. 78519
10. Sari DCR, Aswin S, Susilowati R, Ar-Rochmah M, Prakosa D, Romi M, *et al.* Ethanol extracts of *Centella asiatica* leaf improves memory performance in rats after chronic stress via reducing nitric oxide and increasing brain-derived neurotrophic factor. GSTF J of Psychol 2014; (1):1. https://doi.org/10.7603/s40790-014-0009-0
11. Farhana KM, Malueka RG, Wibowo

S, Gofir A. Effectiveness of Gotu Kola extract 750 mg and 1000 mg compared with folic acid 3 mg in improving vascular cognitive impairment after stroke. Evid Based Complement Alternat Med 2016: 1-6. https://doi.org/10. 1155/2016/2795915
12. Karting T. Clinical application of *Centella asiatica* (L) Urb. In: Herbs, spices, and medicinal plants (Cracker LE, Simon JE Eds.). Phoenix, AZ, USA: Oxyx Press. 1998: 145-73.
13. Chivapat S, Chavalittumrong P, Attawish A, Boonruad T, Bansiddhi J, Phadungpat S, *et al.* Toxicity study of *Centella asiatica* (L) urban. J Thai Trad Alternat Med 2004; 2: 3-17.
14. Perrone D, Ardito F, Giannatempo G, Dioguardi M, Troiano G, Lo Russo L, *et al.* Biological and therapeutic activities, and anticancer properties of curcumin. Expert Ther Med 2015;10(5):1615-23. https://doi. org/10. 3892/etm. 2015. 2749
15. Sharifi-Rad J, Rayess YE, Rizk AA, Sadaka C, Zgheib R, Zam W, *et al*. Turmeric and its major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. Front Pharmacol 2020 15; 11: 01021. https://doi.org/10.3389/fphar.2020. 01021
16. Xu Y, Lin D, Li S, Li G, Shyamala SG, Barish PA, *et al.* Curcumin reverses impaired cognition and neuronal plasticity induced by chronic stress. Neuropharmacology 2009; 57(4):463-71. https://doi.org/10.1016/j.neuropharm.2009.06.010
17. Wang Y, Yin H, Li J, Zhang Y, Han B, Zeng Z, *et al.* Amelioration of β-amyloid-induced cognitive dysfunction and hippocampal axon degeneration by curcumin is associated with suppression of CRMP-2 hyperphosphorylation. Neurosci Lett 2013; 557:112-7. https://doi.org/10.1016/j.neulet.2013.10.024
18. Yin HL, Wang YL, Li JF, Han B, Zhang XX, Wang YT, *et al.* Effects of curcumin on hippocampal expression of NgR and axonal regeneration in Aβ-induced cognitive disorder rats. Genet Mol Res 2014; 13(1):2039-47. https://doi.org/10.4238/2014.March.24.8
19. Baum L, Ng A. Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's Disease animal models, J Alzheimer Dis 2004; 6 (4):367-77. https://doi.org/10.3233/JAD-2004-6403
20. McClure R, Ong H, Janve V, Barton S, Zhu M, Li B, *et al.* Aerosol delivery of curcumin reduced amyloid-deposition and improved cognitive performance in transgenic model of Alzheimer's disease. J Alzheimer Dis 2016; 55 (2): 797-811. https://doi. org/10. 3233/JAD-160289
21. Kim SH & Lee HS. Acute oral toxicity study of ethanol extract of *Curcuma longa* L. in mice. J Life Sci 2014; 24(10): 1132-36 https://doi.org/10.5352/JLS.2014.24.10.1132
22. Handayani ES, Nurmasitoh T, Ahmad SA, Fauziah AN, Rizam R, Rahmawati RY, *et al.* Effect of BCCAO duration and animal models sex on brain ischemic volume after 24 hours reperfusion. Bangladesh J Med Sci 2018; 17(1): 129-37. https://doi.org/10.3329/bjms.v17i1.35293
23. Jokinen H, Melkas S, Yikoski R, Pohjasvaara T, Kaste M, Erkinjuntti, *et al.* Post-stroke cognitive impairment is common even after successful clinical recovery. Eur J Neurol, 2015; 22: 1288-94. https://doi.org/10.1111/ene.12743
24. Kraeuter AK, Guest PC, and Sarnyai Z. The Y-Maze for assessment of spatial working and reference memory in mice. Methods Mol Biol 2019;1916:105-111. https://doi.org/10.1007/978-1-4939-8994-2_10
25. Sbrini G, Brivio P, Fumagalli M, Giavarini F, Caruso D, Racagni G, *et al*. *Centella asiatica* l. Phytosome improves cognitive performance by promoting bdnf expression in rat prefrontal cortex. Nutrients 2020; 12 (2): 355. https://doi. org/10. 3390/nu12020355
26. Sari DCR, Arfian N, Tranggono U,

Setyaningsih WAW, Romi MM, Emoto N. *Centella asiatica* (Gotu kola) ethanol extract up-regulates hippocampal brain-derived neurotrophic factor (BDNF), tyrosine kinase B (TrkB) and extracellular signal-regulated protein kinase 1/2 (ERK1/2) signaling in chronic electrical stress model in rats. Iran J Basic Med Sci 2019; 22(10): 1218.

27. Wang R, Li YB, Li YH, Xu Y, Wu HL, Li XJ. Curcumin protects against glutamate excitotoxicity in rat cerebral cortical neurons by increasing brain-derived neurotrophic factor level and activating TrkB. Brain Res 2008; 1210: 84-91. https://doi.org/10.1016/j.brainres.2008.01.104
28. Sbrini G, Brivio P, Sangiovanni E, Fumagalli M, Racagni G, Dell'Agli M, *et al.* Chronic treatment with a phytosomal preparation containing *Centella asiatica* L. and *Curcuma longa* L. affects local protein synthesis by modulating the BDNF-mTOR-S6 pathway. Biomedicines 2020; 8(12):544. https://doi.org/10.3390/biomedicines8120544
29. Palpu P, Rao CV, Kishore K, Gupta YK, Kartik R, Govindrajan R. U. S. Patent No. 7,429,397. Washington, DC: U. S. Patent and Trademark Office. 2008.
30. Astuti, Sutarni S, and Setyopranoto I. Serum brain-derived neurotrophic factor (BDNF) level may predict the functional outcome of acute ischemic stroke patients. Biomed Pharmacol J 2020; 13:4. https://doi. org/10. 13005/bpj/2075
31. Hassan TM, Yarube IU. Peripheral brain-derived neurotrophic factor is reduced in stroke survivors with cognitive impairment. Pathophysiology 2018; 25(4):405-10. https://doi.org/10.1016/j.pathophys.2018.08.003
32. Hsu CC, Kuo TW, Liu WP, Chang CP, Lin HJ. Calycosin preserves BDNF/TrkB signaling and reduces post-stroke neurological injury after cerebral ischemia by reducing accumulation of hypertrophic and TNF-α-containing microglia in rats. J Neuroimmune Pharmacol 2020; 15 (2): 326-39. https://doi,org/10.1007/s11481-019-09903-9.
33. Zhang ZH, Wu LN, Song JG, Li WQ. Correlations between cognitive impairment and brain derived neurotrophic factor expression in the hippocampus of post-stroke depression rats. Mol Med Rep 2012; 6(4): 889-93. https://doi.org/10.3892/mmr.2012.1009
34. Luo, L, Li, C, Du, X, Shi, Q, Huang, Q, Xu X, *et al.* Effect of aerobic exercise on BDNF/proBDNF expression in the ischemic hippocampus and depression recovery of rats after stroke. Behav Brain Res 2019; 362: 323-31. https://doi.org/10.1016/j.bbr.2018.11.037